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Isolation and characterization of mutants of the facultative methylotroph *Arthrobacter* P1 blocked in one-carbon metabolism

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Abstract. Among methylamine and/or ethylamine minus mutants of *Arthrobacter* P1 four different classes were identified, which were blocked either in the methylamine transport system, amine oxidase, hexulose phosphate synthase or acetaldehyde dehydrogenase. The results indicated that a common primary amine oxidase is involved in the metabolism of methylamine and ethylamine. Growth on ethylamine, however, was not dependent on the presence of the methylamine transport system. In mutants lacking amine oxidase, methylamine was unable to induce the synthesis of hexulose phosphate synthase, thus confirming the view that the actual inducer for the latter enzyme is not methylamine, but its oxidation product formaldehyde. Contrary to expectation, when the formaldehyde fixing enzyme hexulose phosphate synthase was deleted (mutant Art 11), accumulation of formaldehyde during growth on choline or on glucose plus methylamine as a nitrogen source did not occur. Evidence was obtained to indicate that under these conditions formaldehyde may be oxidized to carbon dioxide via formate, a sequence in which peroxidative reactions mediated by catalase are involved. In addition, a specific NAD-dependent formaldehyde dehydrogenase was detected in choline-grown cells of wild type *Arthrobacter* P1 and strain Art 11. This enzyme, however, does not play a role in methylamine or formaldehyde metabolism, apparently because these compounds do not induce its synthesis.

Key words: *Arthrobacter* P1 – Methylamine – Methylotrophy – Regulation – RuMP cycle of formaldehyde fixation – Mutants – Amine oxidase – Hexulose phosphate synthase – Formaldehyde dehydrogenase – Choline

In many methylotrophic bacteria the ribulose monophosphate (RuMP) cycle for formaldehyde fixation functions as a primary carbon assimilation pathway. Via this cycle the conversion of three formaldehyde molecules into a C_3-compound, glyceraldehyde-3-phosphate or pyruvate, is accomplished (Anthony 1982). Most RuMP cycle organisms currently under investigation are obligate methylotrophs which are only able to grown on C_1-compounds, although in some cases a very limited range of heterotrophic substrates is also utilized (Levering 1985). For unknown reasons the isolation of mutants of these organisms has turned out to be very difficult (Holloway 1984). We have therefore adopted *Arthrobacter* P1, a Gram-positive facultative methylotroph, able to grow on methylated amines (but not on methanol) and a variety of "heterotrophic" substrates, as a model organism for studies on the regulation of the RuMP cycle (Levering et al. 1981a). Attempts to develop methods for the isolation of mutants of this versatile bacterium were successful and, when using UV-irradiation, a variety of mutants was readily obtained (Dijkhuizen et al. 1981).

Studies of enzyme profiles in wild type *Arthrobacter* P1 revealed that the synthesis of at least five enzymes is specifically induced during growth on methylamine. These enzymes are an amine transport system (Dijkhuizen et al. 1982), amine oxidase, hexulose phosphate synthase (HPS), hexulose phosphate isomerase (HPI) (Levering et al. 1981a; 1982) and transaldolase (Levering and Dijkhuizen 1986). The first two enzymes are involved in the uptake of methylamine into the cell and its oxidation into formaldehyde and ammonia, whereas HPS and HPI are the unique steps of the RuMP cycle (Fig. 1). In *Arthrobacter* P1 transaldolase plays an important role in both the pentose phosphate pathway and in the rearrangement reactions of the RuMP cycle, serving to regenerate the formaldehyde-acceptor molecule ribulose monophosphate. Recently (Levering and Dijkhuizen 1986), we reported the presence of two isoenzymes of transaldolase in this organism. One of these enzymes is synthesized constitutively, whereas the other is specifically induced during growth on methylamine. Synthesis of these five enzyme systems, however, is not restricted to growth on methylamine (Fig. 1). The activities of the methylamine transport system and amine oxidase are also detectable during growth on ethylamine (Dijkhuizen et al. 1982; Levering et al. 1981a, 1984). Synthesis of HPS, HPI and the inducible transaldolase on the other hand is also observed during growth on choline, the metabolism of which results in the intermediary production of formaldehyde (Levering et al. 1981b; Levering and Dijkhuizen 1986). These observations and the data reported by Levering et al. (1986a, b) have led to the conclusion that in *Arthrobacter* P1 the synthesis of these five enzymes is not regulated co-ordinately but induced sequentially, by lower aliphatic primary amines (amine transport system, amine oxidase) and formaldehyde (HPS, HPI, transaldolase).

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Abbreviations. RuMP, ribulose monophosphate; HPS, hexulose phosphate synthase; HPI, hexulose phosphate isomerase

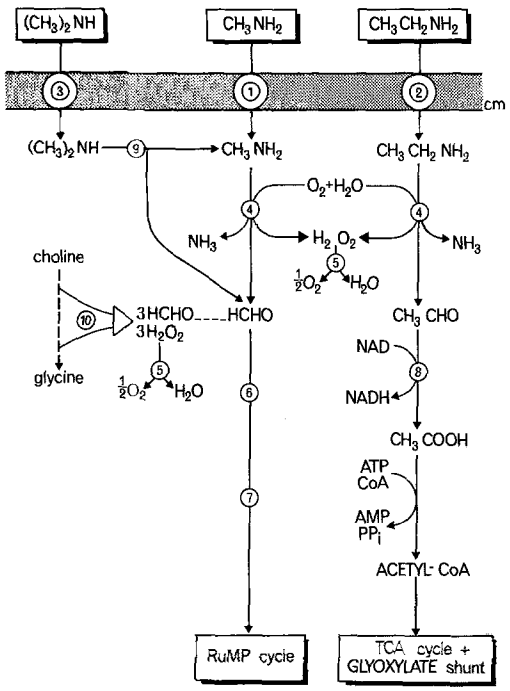

Fig. 1. A schematic representation of the metabolism of some amines, aldehydes and choline in *Arthrobacter* P1. (*1*) Methylamine (*2*) ethylamine and (*3*) dimethylamine transport systems; (*4*) amine oxidase; (*5*) catalase; (*6*) hexulose phosphate synthase (HPS); (*7*) hexulose phosphate isomerase (HPI); (*8*) acetaldehyde dehydrogenase; (*9*) dimethylamine monooxygenase; (*10*) choline metabolism

Our current studies are aimed at the elucidation of the regulation of the synthesis of these enzymes at the molecular level. In this paper we report the isolation and biochemical characterization of amine-negative mutants of *Arthrobacter* P1.

Materials and methods

Organism. Arthrobacter P1 NCIB 11625 and its maintenance have been described previously (Levering et al. 1981a). Mutants of *Arthrobacter* P1 and strain Art 11 were isolated following UV light (254 nm) irradiation (3.5 $W \cdot m^{-2}$, for 20, 45, 60 or 90 s) as described by Dijkhuizen et al. (1981). Among the survivors of this UV treatment strains were selected that had lost the ability to grow on methylamine, ethylamine or acetate agar plates. These strains were maintained on plates containing 1% (w/v) yeast extract and 1.2% (w/v) agar.

Media and cultivation. Arthrobacter P1 wild type and mutant strains were grown at 30° C in 1-l conical flasks containing 250 ml of the mineral salts medium described by Levering et al. (1981a).

Solutions of carbon sources were added to final concentrations of 10, 25 or 40 mM as indicated. Ethylammonium chloride, dimethylammonium chloride, choline, betaine, dimethylglycine and sarcosine were filter sterilized. All other carbon sources were heat-sterilized. Ammonia-free mineral salts medium was prepared as described by Levering and Dijkhuizen (1985). This medium was supplemented with one of the amines (25 mM) as the nitrogen source and glycerol as the carbon source. Incubation was on a rotary shaker at 200 rev./min. Growth on formaldehyde was in formaldehyde-limited continuous cultures (Levering et al. 1986a) in the medium described by Levering and Dijkhuizen (1985). In the experiments involving continuous addition of formaldehyde (Levering et al. 1986b) the organisms were grown in a batch fermenter (Harder et al. 1974) with a working volume of 3 l. Substrate transition experiments were performed as described by Levering and Dijkhuizen (1986).

Growth of the organism was monitored by measuring the absorbance of the culture at 433 nm in a Vitatron 280 colorimeter (Vitatron, Dieren, The Netherlands). Cells for enzyme analysis were harvested before they had reached the stationary growth phase, by centrifugation at $6{,}000 \times g$ for 10 min at 4° C, washed once with 50 mM potassium phosphate buffer pH 7.0 containing 5 mM $MgSO_4$ and resuspended in this buffer to a concentration of 5 – 10 mg dry weight/ml. These suspensions were used immediately for enzyme assays or stored at −20° C until required.

Preparation of extracts and enzyme assays. Preparation of cell-free extracts and assays of activities of C_1-specific enzymes were according to Levering et al. (1981a, b; 1982) and Dijkhuizen et al. (1982). Acetaldehyde dehydrogenase (NAD-dependent) (EC 1.2.1.–) was assayed as described by Levering et al. (1984); isocitrate lyase (EC 4.1.3.1) according to Dixon and Kornberg (1959), except that 50 mM imidazol-HCl pH 7.5 was used as a buffer; formate dehydrogenase (NAD-dependent) (EC 1.2.1.2) according to Johnson et al. (1964) and formate dehydrogenase (NAD-independent) (EC 1.2.1.–) as described by Dijkhuizen et al. (1979).

Formaldehyde dehydrogenase (NAD-dependent) (EC1.2.1.–). The reaction mixture (1 ml) contained: potassium phosphate buffer pH 8.0, 50 µmol; magnesium chloride, 2.5 µmol; NAD, 0.4 µmol; potassium cyanide, 5 µmol, and extract. The reaction was started by the addition of 5 µmol formaldehyde.

Substrate/product analyses. Formaldehyde was assayed with the method of Nash (1953); formate according to Lang and Lang (1972), and methylamine as described by Levering et al. (1984).

Protein and dry weight determinations. Protein was determined by the method of Lowry et al. (1951), using bovine serum albumin as a standard. Dry weight of bacterial suspensions was determined with a carbon analyzer (Beckman, model 915A), connected to an infrared analyzer (Beckman, model 865). Carbon contents were multiplied by a factor of 2 to obtain dry weight.

Results

Isolation and identification of amine-negative mutants. Following UV-irradiation of wild type *Arthrobacter* P1, approximately 0.5% of the colonies tested in the initial screening appeared to be affected in amine metabolism. In further steps 10% of these colonies turned out to be stable mutants. Table 1 lists representatives of the various classes of mutants that were distinguished on the basis of their ability to grow in minimal medium containing methylamine or ethylamine as a carbon- or nitrogen source.

As shown in Table 2, growth of wild type *Arthrobacter* P1 in media containing glycerol/ammonia in the presence

Table 1. Ability of wild type *Arthrobacter* P1 and amine-negative mutants to grow in minimal media on various carbon- and nitrogen sources

Strain	carbon (C)- and nitrogen (N) sources							
	methylamine		ethylamine		dimethylamine		choline	acetate
	C	N	C	N	C	N	C	C
Arthrobacter P1	+	+	+	+	+	+	+	+
Art 11	−	+	+	+	−	+	+	+
Art 37	−	−	+	+	+	+	+	+
Art 38	−	−	−	−	−	−	+	+
Art 76	+	+	−	+	+	+	+	+
Art 106	−	+	−	+	−	+	+	−

The ability to use amines as a nitrogen source was tested in media without ammonium sulphate containing glycerol as the carbon source
+ growth
− no growth

Table 2. Enzyme activities (nmol · min^{-1} · mg^{-1} protein) in whole cells and in cell-free extracts of wild type *Arthrobacter* P1 and amine-negative mutants following growth on glucose (10 mM), succinate (15 mM), or glycerol (25 mM)/ammonium sulphate minimal medium, supplemented with either 25 mM of methylamine (MA), ethylamine (EA) or dimethylamine (DMA), or on choline (10 mM) alone

Strain	Medium		Enzyme			
			[^{14}C]Methylamine transport (whole cells)	Amine oxidase	Hexulose phosphate synthase	Acetaldehyde dehydrogenase
Arthrobacter P1	glucose	+MA	26	223	515	0
	succinate	+MA	−	95	21	0
	glycerol	+MA	115	470[a]	719[a]	0
		+EA	0	260[a]	0	98[a]
		+DMA	30	252	472	0
	choline		0	0	310	0
Art 11	glycerol	+MA	0	340[a]	0[a]	
	choline		0	0	0	
Art 37	glycerol	+MA	0	0	0	
		+EA	0	135	0	
		+DMA	28	151	568	
	choline		0	0	211	
Art 38	glycerol	+MA	<1	0	0	
		+EA	0	0	0	
		+DMA	−	0	0	
	choline		0	0	228	
Art 76	glycerol	+EA[a]	−	188	−	0

[a] Data of Levering et al. (1984)
− not determined

of methylamine, ethylamine or dimethylamine, resulted in induction of the various amine-specific enzymes. In comparison, lower activities of these enzymes were observed following growth under the same conditions with glucose or succinate as a carbon source. As reported previously (Levering et al. 1981a), growth of the organism on choline resulted in synthesis of HPS only. These observations allowed the subsequent identification of the enzyme lesions in the various mutants. The phenotype of strain Art 11 already indicates that the metabolic block in this mutant is in one of the enzymes of the RuMP cycle (Fig. 1; Table 1). An analysis of enzyme activities present in this strain revealed that it is unable to synthesize HPS (Table 2). The failure to detect an active methylamine transport system in Art 11 may reflect an internal conversion of methylamine to formaldehyde which is a known very strong inhibitor of methylamine uptake (Dijkhuizen et al. 1982).

Growth of strain Art 37 in the presence of methylamine did not induce synthesis of the methylamine transport system, amine oxidase or HPS. Amine oxidase activity, however, was clearly detected in cells grown with ethylamine or dimethylamine, while synthesis of HPS occurred during growth in the presence of dimethylamine or choline. From these results we concluded that in Art 37 the block is in the methylamine transport system. The low rate of [^{14}C]methylamine uptake observed with dimethylamine-grown cells is most likely due to the presence of a transport system for this secondary amine which possesses a low affinity for methylamine (Dijkhuizen et al. in preparation). Strain Art 37 is still able to grow on ethylamine (Table 1)

and the results therefore indicate that, at least in this mutant, ethylamine does not enter the cell via the methylamine transport system.

The data obtained with mutant Art 38 indicate that this organism is either amine oxidase-negative or is blocked in the synthesis of both this enzyme and the methylamine transport system. This mutant is unable to use either methylamine, ethylamine or dimethylamine as a carbon- or nitrogen source and these compounds are also unable now to induce synthesis of amine oxidase or HPS. Growth with choline, however, clearly resulted in synthesis of HPS. Consistently, negligible levels of the methylamine transport system were observed in this mutant. The failure of this mutant to grow on dimethylamine remains to be explained.

The enzyme data obtained with mutant Art 76 showed that this organism is blocked in the synthesis of acetaldehyde dehydrogenase. Its incubation in the presence of ethylamine did result in synthesis of amine oxidase which explains its ability to use ethylamine as a nitrogen source, but failed to induce synthesis of acetaldehyde dehydrogenase.

Fate of formaldehyde in mutant strain Art 11. The initial characterization of the HPS-negative strain Art 11 showed that this organism was still able to use methylamine as a nitrogen source during growth with glucose or glycerol, despite the fact that this situation may lead to intracellular accumulation of (toxic) formaldehyde (Fig. 1; Table 1). In addition, strain Art 11 could still grow on choline although the available enzymic evidence (Levering et al. 1981 b) indicated that in the absence of a functional RuMP cycle two molecules of formaldehyde should accumulate per choline molecule oxidized.

In cultures of mutant Art 11, but not with wild type, growing on glucose with methylamine as the sole nitrogen source excretion of small quantities of both formaldehyde and formate was observed. However, the doubling time of Art 11 was similar to that of wild type *Arthrobacter* P1, so that growth inhibitory effects of these compounds were not apparent. Although Art 11 is completely blocked in HPS synthesis, stoichiometric conversion of methylamine-carbon into formaldehyde- plus formate carbon did not occur. At a culture density equivalent to 500 mg dry weight of cells/l, maximum levels of only 0.7 mM formaldehyde and 0.1 mM formate were detected. Based on the elementary composition of cells of *Arthrobacter* P1 ($C_4H_8O_2N$) it can be calculated that in these cultures at least 5 mM of methylamine (CH_3NH_2) had been converted into ammonia and formaldehyde via the amine oxidase reaction. This indicated that formaldehyde was further metabolized, either by its assimilation by way of other pathways or by oxidation via formate to carbon dioxide. This was even more evident during growth of Art 11 on choline since this did not result in accumulation of formaldehyde or formate at all. One possible explanation which was considered is that in the absence of HPS activity Art 11 employs a cyclic serine pathway for the regeneration of the formaldehyde acceptor molecule glycine during growth on choline (Levering et al. 1981 b). If this were the case then the glyoxylate cycle enzyme isocitrate lyase most likely would play an important role (Anthony 1982). The activity of isocitrate lyase was indeed considerably higher in choline-grown cells of Art 11 than in wild type cells (50 and 7 nmol · min^{-1} · mg^{-1} protein, respectively). We therefore set out to isolate mutants of Art

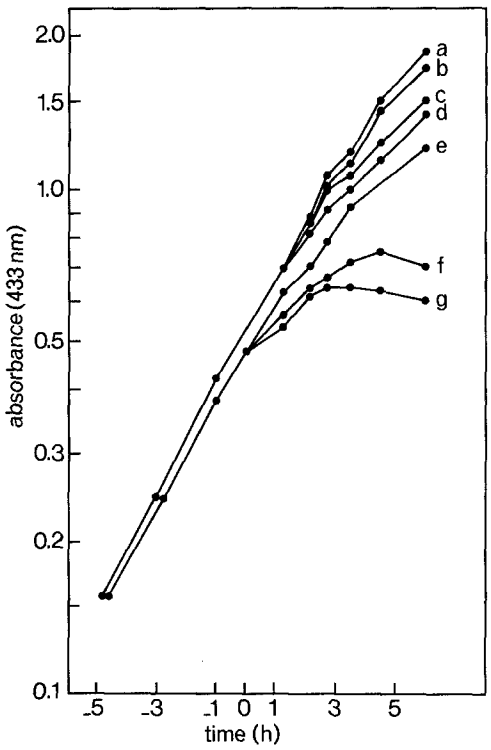

Fig. 2. Effect of addition of aminotriazole (AT) (arrow) to cultures of *Arthrobacter* P1 (*a*–*d*) and Art 11 (*e*–*g*), growing on choline (10 mM). *a, e,* no addition; *b, f,* 0.5 mM AT; *c, g,* 1.0 mM AT; *d,* 3.0 mM AT

11 unable to grow on acetate and selected isocitrate lyase-negative double mutants. The phenotype of one of these mutants, strain Art 106, is shown in Table 1. In addition to the features displayed by strain Art 11 it is now also unable to grow on the C_2 substrates acetate and ethylamine. However, Art 106 and similar mutants were still able to grow on choline, thus ruling out a possible role for a cyclic serine pathway in the fixation of formaldehyde.

Formaldehyde may also be oxidized by way of peroxidative reactions catalyzed by catalase (in the presence of hydrogen peroxide generated in the amine oxidase reaction or the conversion of choline to glycine; Fig. 1) or via an aldehyde dehydrogenase. High levels of catalase have been reported previously (Levering et al. 1981 a) in methylamine- and choline-grown cells of *Arthrobacter* P1. Comparable activities of this enzyme were observed in the present study in cells of strain Art 11. Evidence for a possible role of catalase in removing formaldehyde and formate were sought in studies with aminotriazole, which is known to inhibit catalase irreversibly (Cohen and Somerson 1969; Van Vliet-Smits et al. 1981). Addition of 0.5 mM of this compound to cells of Art 11, growing exponentially on choline (Fig. 2) or glycerol/methylamine, completely inhibited growth within a few hours. Growth of wild type *Arthrobacter* P1 on the other hand was hardly affected even in the presence of 3 mM aminotriazole (see also Levering et al. 1981 a).

Levering et al. (1984) observed that the acetaldehyde dehydrogenase synthesized during growth on ethylamine also displays a low affinity for formaldehyde. This enzyme, however, is only induced by acetaldehyde and not by the formaldehyde produced from either methylamine or choline (Table 2). Until now no evidence had been obtained to

Table 3. Specific activities (nmol · min^{-1} · mg^{-1} protein) of hexulose phosphate synthase and formaldehyde dehydrogenase (NAD-dependent) in wild type *Arthrobacter* P1 during growth on various substrates

Growth substrate	Hexulose phosphate synthase	Formaldehyde dehydrogenase (NAD-dependent)
Methylamine	808[a]	0
Formaldehyde	1830[b]	0
Choline	310	73 (78[c]) (70[d])
Betaine	341[a]	75
Dimethylglycine	244	86
Sarcosine	71[a]	19
Glycine	0[a]	0
Serine	0	0

[a] Data of Levering et al. (1981 b)
[b] Data of Levering et al. (1986a)
[c] Activity in strain Art 11
[d] Activity in strain Art 76

indicate the involvement of a specific formaldehyde dehydrogenase in the metabolism of C_1 compounds in *Arthrobacter* P1. Instead, the available enzymatic evidence suggested that, as in many other RuMP cycle methylotrophs, formaldehyde was solely oxidized to carbon dioxide via the dissimilatory RuMP cycle (Levering et al. 1981 a). We again observed that this is the case during growth of *Arthrobacter* P1 and Art 11 with methylamine, but growth of the organisms with choline clearly resulted in the synthesis of a specific formaldehyde dehydrogenase (Table 3). Further studies also revealed the presence of formaldehyde dehydrogenase activity in cells of *Arthrobacter* P1 grown on betaine, dimethylglycine or sarcosine, intermediates in choline metabolism, but not in cells grown on glycine or serine. Synthesis was also not observed in formaldehyde-grown cells of *Arthrobacter* P1. NAD-(in)dependent formate dehydrogenase activities were not detected under any growth condition and methylamine- or choline-grown cells showed negligible formate-oxidizing capacities. The presence of formaldehyde dehydrogenase activity in choline-grown *Arthrobacter* P1 but not in methylamine-grown cells was rather puzzling at first. The regulation of the synthesis of this enzyme was therefore investigated in substrate-transition experiments in which cultures were transferred from methylamine to choline containing media and vice versa.

Regulation of formaldehyde dehydrogenase synthesis. Following incubation of methylamine-grown cells of *Arthrobacter* P1 in media with choline as the carbon- and energy source, growth started after 2 h to reach a doubling time of 2.1 h (Fig. 3). An analysis of enzyme activities in cell-free extracts showed that choline oxidase and formaldehyde dehydrogenase were undetectable in the inoculum cells, but they were synthesized in the presence of choline. On the other hand the activity of amine oxidase decreased rapidly and almost reached zero levels at $t = 11$ h. The same response was observed for HPS, but the strong decrease in the activity of this enzyme levelled off, at $t = 7$ h, to reach a value of approximately 400 nmol · min^{-1} · mg^{-1} protein. This HPS activity is routinely found in choline-grown cells of the organism (Tables 2 and 3).

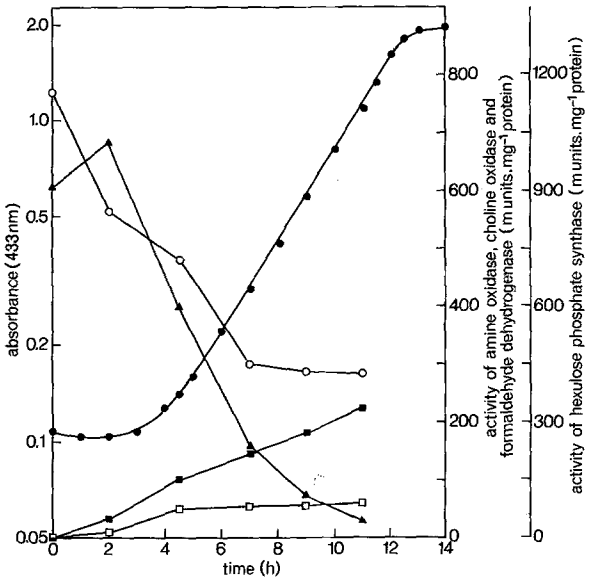

Fig. 3. Methylamine-choline (10 mM) substrate transition experiment with *Arthrobacter* P1. ●, absorbance; ○, hexulose phosphate synthase; ▲, amine oxidase; ■, choline oxidase; □, formaldehyde dehydrogenase

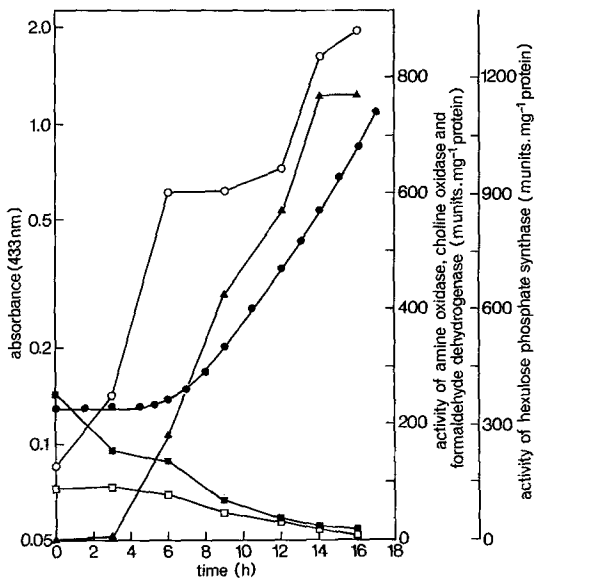

Fig. 4. Choline-methylamine (40 mM) substrate transition experiment with *Arthrobacter* P1. ●, absorbance; ○, hexulose phosphate synthase; ▲, amine oxidase; ■, choline oxidase, □, formaldehyde dehydrogenase

After incubation of choline-grown cells of *Arthrobacter* P1 in methylamine containing media, a lag phase of 5 h was observed before growth started to reach a doubling time of 3.0 h (Fig. 4). During this lag phase the enzyme HPS, already present in choline-grown cells, rapidly increased in activity but growth only resumed once the synthesis of amine oxidase had started. Choline oxidase and formaldehyde dehydrogenase on the other hand decreased in activity and almost reached zero levels at $t = 16$ h. These results indicate that synthesis of formaldehyde dehydrogenase is switched off when choline-grown cells are transferred into methylamine-containing media.

Further evidence for the in vivo role of the formaldehyde dehydrogenase in oxidizing formaldehyde was sought in experiments in which *Arthrobacter* P1 and strain Art 11 were grown in batch cultures on choline with, once the mid-exponential growth phase had been reached, a continuous addition of formaldehyde at a low constant rate (see Levering et al. 1986b). As was the case with cells of *Arthrobacter* P1 growing on glucose or acetate (Levering et al. 1986b) addition of formaldehyde (1 mmol $\cdot l^{-1} \cdot h^{-1}$) to a culture growing exponentially on choline resulted in the almost complete consumption of formaldehyde and a rapid 3.5-fold increase in the HPS levels (from 361 to 1290 nmol $\cdot$ min$^{-1} \cdot$ mg^{-1} protein). However, the level of the NAD-formaldehyde dehydrogenase remained virtually constant (99–120 nmol $\cdot$ min$^{-1} \cdot$ mg^{-1} protein). Addition of formaldehyde at a rate of 1 or 0.5 mmol $\cdot l^{-1} \cdot h^{-1}$ to cultures of strain Art 11 growing exponentially on choline resulted in accumulation of formaldehyde and almost immediate cessation of growth. However, growth continued normally when formaldehyde was added to such a culture at a rate of 0.25 mmol $\cdot l^{-1} \cdot h^{-1}$. Under these conditions formaldehyde remained virtually undetectable but instead formate accumulated (1.3 mM after 8 h). Again in this experiment the level of the NAD-formaldehyde dehydrogenase did not change significantly. Comparable results were obtained following addition of formaldehyde (or methylamine) to the medium reservoir of a choline-limited (S_R = 7.5 mM) continuous culture ($D = 0.10\ h^{-1}$) of strain Art 11. Only at S_R values of 6 mM formaldehyde, or below, steady states were obtained, again with virtually unchanged levels of the formaldehyde dehydrogenase compared to growth on choline alone (70 nmol $\cdot$ min$^{-1} \cdot$ mg^{-1} protein). Formaldehyde and formate remained undetectable in the culture supernatants. Higher concentrations of formaldehyde (or methylamine) in the medium reservoir resulted in wash-out of the cultures (or accumulation of methylamine). Previously (Levering et al. 1986a) we reported that wild type *Arthrobacter* P1 can be grown on formaldehyde as the sole carbon- and energy source in formaldehyde-limited (S_R = 25 mM) continuous cultures up to dilution rates of at least 0.20 h^{-1}. In these cultures the formaldehyde-fixing enzyme HPS had a very high activity but the NAD-formaldehyde dehydrogenase was undetectable (Table 3).

These observations indicate that, although formaldehyde is an important intermediate in the metabolism of both methylamine and choline, the enzyme formaldehyde dehydrogenase is only involved in choline utilization. The apparent reason for this is that synthesis of the enzyme is only induced during growth with choline, and on intermediates derived from it (betaine, dimethylglycine and sarcosine), but not with methylamine or formaldehyde (Table 3).

Discussion

Compared to the situation observed with obligate RuMP cycle methylotrophs (Holloway 1984), the isolation of mutants of *Arthrobacter* P1 blocked in C_1 metabolism is relatively simple. Using UV-irradiation a variety of mutants unable to use amines as carbon- and/or nitrogen source were readily obtained. Four different classes of amine-negative mutants have been identified. These are blocked in methylamine transport, amine oxidase, hexulose phosphate synthase or acetaldehyde dehydrogenase synthesis, respectively. At the moment the precise nature of these mutations, i.e. whether they are in regulatory or structural genes, is unknown. The availability of these mutants will greatly facilitate a further analysis of the organization and regulation of the various genes involved at the molecular level.

In previous studies on the properties of amine oxidase in methylamine- and ethylamine-grown cells of *Arthrobacter* P1 (Van Vliet-Smits et al. 1981; Levering et al. 1984) evidence was obtained that an identical enzyme is involved in the conversion of both primary amines into the respective aldehyde. The characterization of strain Art 38, which is unable to use either methylamine or ethylamine, as an amine oxidase-negative mutant confirmed this view. Apparently both substrates can induce synthesis of this enzyme. With respect to the uptake of these amines into the cell the situation appears to be more complex since strain Art 37 which is blocked in the methylamine transport system, is unimpaired in ethylamine metabolism. Previously we observed that ethylamine-grown cells of wild type *Arthrobacter* P1 are able to metabolize methylamine immediately (Levering et al. 1984). The available data therefore indicate that in wild type cells ethylamine either also induces the specific methylamine transport system, or that a specific ethylamine transport system is induced which has affinity for both amines. Strain Art 38, blocked in amine oxidase, when incubated in the presence of methylamine failed to induce the synthesis of HPS. These results confirm the conclusion drawn on the basis of physiological experiments that formaldehyde produced from methylamine is the actual inducer for HPS (and HPI/transaldolase).

Growth of *Arthrobacter* P1 on choline not only results in synthesis of RuMP cycle enzymes but also of some enzymes of the serine pathway (Levering et al. 1981b). Since *Arthrobacter* P1 is a very flexible organism, the possibility that in the HPS-minus mutant Art 11 formaldehyde was now being fixed via a complete cyclic serine pathway was investigated. However, loss of isocitrate lyase, supposedly a key enzyme in this pathway, did not affect growth of strain Art 106 on choline. We subsequently observed that aminotriazole, a catalase inhibitor, strongly inhibited growth of strain Art 11 on choline and, in addition, synthesis of an NAD-formaldehyde dehydrogenase occurred during growth on choline. This formaldehyde dehydrogenase is different from the acetaldehyde dehydrogenase present in ethylamine-grown cells because strain Art 76, blocked in the acetaldehyde dehydrogenase, is still able to synthesize the formaldehyde dehydrogenase. Further studies are in progress to characterize the newly discovered formaldehyde dehydrogenase. Interestingly, synthesis of this enzyme is *not* stimulated by formaldehyde itself and thus appears to be linked to that of the serine pathway enzymes (Levering et al. 1981b) rather than to the metabolism of methylamine. This formaldehyde dehydrogenase may therefore be considered as a formaldehyde scavenger (Attwood and Quayle 1984) similar to the enzymes encountered in the non-methylotrophic bacteria *Pseudomonas putida* (Ando et al. 1979) and *Rhodococcus erythropolis* (Eggeling and Sahm 1984). The main function of such enzymes appears to be to prevent accumulation of formaldehyde produced during growth on "heterotrophic" compounds containing methyl groups. However, in wild type *Arthrobacter* P1 the formaldehyde-fixing enzyme HPS, which is rapidly induced

up to high levels by formaldehyde itself, appears to be considerably more effective in doing so than the formaldehyde dehydrogenase. The latter enzyme and/or the peroxidative reactions of catalase (Van Dijken et al. 1975) most likely prevent accumulation of toxic formaldehyde in the HPS-negative mutant Art 11 during growth on choline or glucose plus methylamine, respectively. The relative contribution of these systems, during growth on choline, however, remains unclear. Compared to wild type *Arthrobacter* P1, cells of Art 11 growing on choline were very sensitive to the addition of extra formaldehyde both in batch and in continuous cultures. Two possible explanations are currently considered to account for these observations: either insufficient hydrogen peroxide is generated to enable catalase to remove the extra formaldehyde and formate, or the activity of formaldehyde dehydrogenase which is not further induced by formaldehyde is too low to cope efficiently with the extra formaldehyde supplied.

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